

Listing of Claims

1. Canceled

2-11 Withdrawn

Q 12. (Currently amended) A method for modulating expression of a target gene product in a cell ~~in culture~~ that contains a target gene under control of one or more regulatory elements,

said method comprising contacting the cell ~~in culture~~ under suitable conditions with one or more regulatory agents attached to a translocating polypeptide, whereby the one or more regulatory agents are translocated into the cell in culture and interact therein with the one or more regulatory elements, thereby modulating expression of the target gene product by the cell wherein the translocating polypeptide exhibits receptor-independent and energy-free penetration of cell membranes, wherein the cell does not have a cell wall; and wherein the one or more regulatory agents include a recombinase.

13. (Currently amended) The method according to claim 12 wherein the cell ~~in culture~~ is a mammalian, yeast, or insect ~~or plant~~ cell.

14. Canceled

15. (Original) The method according to claim 12 wherein the translocating polypeptide is a VP22 polypeptide, Antp, or Protein H.

16. (Original) The method according to claim 12 wherein the translocating polypeptide is a VP22 polypeptide.

17. Canceled

18. Withdrawn

19. Canceled

20. Canceled

21. (Currently amended) The A method according to claim 20 for modulating expression of a target gene product in a cell that contains a target gene under control of one or more regulatory elements,

Q said method comprising contacting the cell under suitable conditions with one or more regulatory agents attached to a translocating polypeptide, whereby the one or more regulatory agents are translocated into the cell in culture and interact therein with the one or more regulatory elements, thereby modulating expression of the target gene product by the cell wherein the translocating polypeptide exhibits receptor-independent and energy-free penetration of cell membranes, wherein the cell does not have a cell wall; wherein the one or more regulatory elements includes a promoter and wherein the one or more regulatory agent is agents include a polymerase specific for the promoter.

22. (Original) The method according to claim 21 wherein the polymerase is T7 RNA polymerase and the promoter is a T7 promoter.

23. (Currently amended) The A method according to claim 12 for modulating expression of a target gene product in a cell that contains a target gene under control of one or more regulatory elements,

said method comprising contacting the cell under suitable conditions with one or more regulatory agents attached to a translocating polypeptide, whereby the one or more regulatory agents are translocated into the cell in culture and interact therein with the one or more regulatory elements, thereby modulating expression of the target gene product by the cell wherein the translocating polypeptide exhibits receptor-independent and energy-free penetration of cell membranes, wherein the cell does not have a cell wall; wherein the one or more regulatory agent is agents include an HIV Rev protein and the one or more regulatory elements include element is the HIV Rev response element (RRE).

24. Canceled.

25. (Currently amended) The method according to claim 12 wherein the one or more regulatory ~~agent~~ agents and the translocating polypeptide are covalently attached.

26. (Currently amended) The method according to claim 12 wherein the one or more regulatory ~~agent~~ agents and the translocating polypeptide are attached by a linker.

27. (Original) The method according to claim 26 wherein the linker comprises one or more disulfide bonds, salicylhydroxamic acid (SHA), phenylboronic acid (PBA), a SHA-NHS ester, or a combination thereof.

28. (Currently amended) The method according to claim 12 wherein the translocating polypeptide and the one or more regulatory ~~agent~~ agents are units of a fusion protein.

29-30. Withdrawn

31. (Currently amended) The method according to claim 12 wherein the translocating polypeptide and the one or more regulatory ~~agent~~ agents are ~~covalently~~ linked by a biotin-streptavidin complex or the *E. Coli* single stranded DNA binding protein.

32. (Currently amended) The method according to claim 12 wherein the one or more regulatory agents include a site-specific recombinase, the cell ~~line~~ contains a first nucleic acid with at least one site-specific ~~single~~ genomic recombination site, and a plasmid second nucleic acid containing the target gene and at least one site-specific-a recombination site that pairs with the genomic recombination site, and wherein the one or more regulatory agents includes a the recombinase is specific for the paired recombination sites, and wherein translocation of the site-specific recombinase causes recombination between the paired-site-specific recombination sites resulting in stable integration of the target gene into the genome of the cell at the genomic recombination site.

33. (Currently amended) The method according to claim 32 wherein the recombinase is ~~Flp and the recombinase sites are *frt* recombination sites~~ a member of the family of site-specific recombinases selected from the groups consisting of the integrase family of site-specific recombinases and the resolvase/invertase family of site-specific recombinases.

34. (Currently amended) The method according to claim 32 wherein the site-specific recombination sites are *frt* sites and the site-specific recombinase is Flp or the site-specific recombination sites are lox recombination sites and the site-specific recombinase is Cre. ~~recombinase is Cre and the recombinase sites are *lox* recombination sites.~~

35. (Currently amended) The method according to claim 12 wherein the one or more regulatory agents include a site-specific recombinase, wherein the one or more regulatory elements ~~includes a transcription blocking sequence~~ are flanked by site-specific recombinase recombination sites and the regulatory agent is a wherein the site-specific recombinase is specific for the flanking recombination sites, wherein translocation of the site-specific recombinase causes recombination of the flanking site-specific recombination sites, thereby modulating expression of the target gene product.

36. (Currently amended) The method according to claim 35 wherein the ~~recombinase~~ flanking recombination sites are *frt* sites and the site-specific recombinase is Flp or the recombinase flanking recombination sites are *lox* sites and the site-specific recombinase is Cre.

37. Withdrawn

38. (Original) The method according to claim 12 wherein the target gene is a reporter gene.

39. (Original) The method according to claim 12 wherein the target gene is contained within a polynucleotide that further encodes a protein tag.

40. (Original) The method according to claim 12 wherein the target gene encodes a toxic protein.

41. (Original) The method according to claim 39 wherein the protein tag is a myc epitope, a fluorescent peptide, or a poly His tag, or a combination of any two or more thereof.

42-45 Withdrawn

46. Canceled

47-50 Withdrawn

51. (New) The method of claim 12 wherein the cell is a eukaryotic cell.

52. (New) The method according to claim 12 wherein the one or more regulatory agents and the translocating polypeptide are non-covalently attached.

53. (New) The method according to claim 12 wherein the recombinase is a site specific recombinase.

54. (New) The method of claim 53 wherein the site-specific recombinase is a member of a family of site-specific recombinases selected from the group consisting of the integrase family of site-specific recombinases and the resolvase/invertase family of site-specific recombinases.

55. (New) The method according to claim 54 wherein the site-specific recombination sites are *frt* sites and the site-specific recombinase is Flp or the site-specific recombination recombinase sites are *lox* recombination sites and the site-specific recombinase is Cre.

56. (New) A method for modulating expression of a target gene product in a cell that contains a target gene under control of one or more regulatory elements,

Q, said method comprising contacting the cell under suitable conditions with one or more regulatory agents attached to a translocating polypeptide, whereby the one or more regulatory agents are translocated into the cell in culture and interact therein with the one or more regulatory elements, thereby modulating expression of the target gene product by the cell wherein the translocating polypeptide exhibits receptor-independent and energy-free penetration of cell membranes, wherein the cell does not have a cell wall; and wherein the one or more regulatory agents include a DNA-binding protein.

57. (New) The method according to claim 56 wherein the regulatory element is a promoter.

58. (New) The method according to claim 56 wherein the DNA-binding protein has a DNA-binding domain selected from the group consisting of a DNA-binding domain of a member of the steroid/thyroid hormone nuclear receptor superfamily, the GAL4DNA binding domain, and a DNA-binding domain of a Tet operon.

59. (New) The method of claim 58 wherein the DNA-binding domain is that of SeqID No:4.

60. (New) The method according to claim 56 wherein the DNA-binding protein is a histone 1(H1) protein or a non-histone protein HMG-17.

61. (New) The method according to claim 56 wherein the DNA-binding protein is a DNA topoisomerase.

62. (New) The method according to claim 56 wherein the DNA-binding protein has a DNA-binding domain selected from the group consisting of the

Q1
Answer

DNA-binding domains of homeobox proteins, zinc finger proteins, hormone receptors, helix-turn-helix proteins, basic-Zip proteins, and β -ribbon factors.--
